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ROCHESTER UNIV N Y SCHOOL OF MEDICINE AND DENTISTRY
MICROWAVES AND HUMAN LEUKOCYTE FUNCTION.(U)

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AFOSR-80-0111

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AFOSR-TR-81-0537

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Final

TECHNICAL REPORT Jan. 1980 - Feb. 1981

U.S. Air Force Office of Scientific Research, Grant No. AFOSR-80-0111

MICROWAVES AND HUMAN LEUKOCYTE FUNCTION

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This work was also supported in part by funds provided by the U.S. Environmental Protection Agency (R806390).

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1. INTRODUCTION

The objective of these studies is to determine whether exposure to microwaves affects the function of human leukocytes in the resting state and during challenge. Further, if effects on cell function are identified, the studies will attempt to determine whether the effects are likely to enhance or compromise host defense.

Details of background information and methodology of these studies were outlined in the original proposal. Recent reviews are available regarding hematologic and immunologic effects of nonionizing electromagnetic radiation [see Smialowicz, RJ: Bull. N.Y. Acad. Med. 55:1094-1118, 1979] and effects of temperature on immunological responses and host defense [see Roberts, NJ: Microbiol. Rev. 43:241-259, 1979]. Many past studies have used animal models, and may not appropriately reflect events for humans exposed to microwaves.

The initial studies of this project include construction and calibration of a waveguide exposure system, and exposure of human mononuclear leukocytes to 2450 MHz (CW) microwave energy, using low averaged SAR (see Section 4, Studies Planned-Year 02), with determinations of DNA, RNA and protein synthesis.

2. PROGRESS REPORT

This report is divided into two major sections: (a) accomplishments, and (b) problems encountered.

(a) Accomplishments

The accomplishments in year 01 may be listed as follows: 1) design, acquisition and construction of a microwave exposure system; 2) dosimetry analysis of the system; and 3) training of technical staff and quality control assessment.

(i) Upon receipt of funds, Drs. Roberts and Lu visited the laboratory of Dr. H. Ho at the Bureau of Radiological Health, Bethesda, Maryland. Dr. Ho was a designer of the waveguide system judged most appropriate for studies of the proposal, and served as consultant for determination of specific waveguide equipment appropriate for these studies. Upon determining the appropriate instrumentation (see also Section b, "Problems Encountered", below), the equipment was ordered and the system constructed as shown in the Appendix, Figures 1 and 2. All of the equipment was received, with the exception of the amplifier, by late June, 1980. The instruments were tested. For initial calibration (Appendix Sect I-VII), an alternate (borrowed) power source was used. (The project's signal generator produced insufficient power-in the absence of the amplifier-at the sample site after passage through the delivery system). The amplifier was ordered after receipt of supplementary funds from the AFOSR, and arrived on October 20, 1980. Additional comments appear in Section b, "Problems Encountered", below.

(ii) The waveguide microwave exposure system equipment was tested. Analyses of dosimetric and thermal characteristics are provided in the Appendix (pages 7-29). These analyses constitute the major scientific effort achieved within the report period, after system procurement.

(iii) The technician staff from Radiation Biology and Biophysics (RBB) and from Medicine were trained in the alternate procedures during acquisition and calibration of the system, to allow optimum use of personnel. For example, Ms. Lebda (RBB technician) is now trained in tissue culture techniques and qualified to perform the immunological assays of the proposal. Consistent quality of effort has been assured.

(b) Problems Encountered

Certain of the problems encountered in year 01 are described in part to document efforts and, in part, for use of Project Officers in evaluating future proposals (especially in regard to efforts required to construct such a system) and for use in forewarning other investigators of potential problems. Many of the problems produced substantial delays.

(i) The specialized equipment required for construction of a waveguide exposure facility was available only with substantial delay, even with citation of a Department of Defense priority rating assigned to the project. Certain equipment was either modified (incubator with access hole), or intermittently constructed per orders (Vitek or "Bowman" nonperturbing probe), which caused delays. Other equipment (Hewlett-Packard Power Meter) required warranty repair upon testing.

(ii) Economic factors substantially affected the project. The cost of equipment increased, beyond prediction, between the time of submitting the initial proposal and the time by which funds were received (and orders could be placed). This problem was offset by receipt of supplementary funds, but acquisition of the amplifier was delayed.

(iii) Miscellaneous illustrative problems were noted. An appropriate cell culture vessel was needed: it must fit into the waveguide, allow insertion of the temperature probe, allow agitation, maintain sterility, and be economically feasible and hopefully commercially available. Enquiry and testing determined that the appropriate culture vessel was a hybrid (caps of Falcon 2054 plastic tubes combined with glass vials of Rochester Scientific, R761B) from commercially available materials, avoiding the costs and limitations of specially ordered supplies.

The planned agitation of cell cultures during microwave exposure (in order to prevent development of "hot spots") presented special problems. The intra-incubator agitation for the waveguides was initially obtained by positioning of the waveguides within the incubator upon a variable speed, variable stroke, heavy duty Eberbach shaker. However,

the minimal heat generated by the motor during shaker operation was sufficient to raise the temperature within the water-jacketed incubator by 3°C or more (to 40°C or more) within two hours. Thus, a system had to be constructed for internal (within the incubator) agitation, powered externally. This was accomplished by use of shelves that slide on roller bearings within plexiglass guides, moved by a metal rod connected via the access hole in the external Eberbach shaker. The weight and leverage aspects of the connection required adjustments, after an initial connecting rod snapped off at a stressed joint.

3. CAPABILITIES OF THE FACILITIES

(a) Exposure Facilities

The power source has a frequency range of 2.0-4.0 GHz. Amplitude modulation from DC to 1 MHz is available. This would permit us to scan frequencies of 7, 14, 28, 45, 70 and 75 Hz to search for frequency windows. Pulse modulation is available from 50 Hz to 50 KHz.

(b) Immunological Facilities

The following assays of human leukocyte function are performed in our laboratories currently: analysis of lymphocyte DNA, RNA and protein synthesis, monocyte-macrophage accessory cell function for lymphocyte transformation responses, cytotoxicity assays, production of (and response to) lymphokines (e.g., LIF) and monokines (e.g., interferon), phagocytosis and bactericidal activity of mononuclear and polymorphonuclear phagocytes, leukocyte migration assays.

The principal investigator has also been developing flow cytometric analyses of human leukocytes (e.g., for transmembrane potential changes, surface antigen expression, cell cycle analysis and transformation response, etc.) using a multiparameter cell sorter in collaboration with Dr. Paul K. Horan [see Horan PK & Wheelless LL: Science 198:149-157, 1977]. The methodology allows single cell flow analysis (and sorting) instead of "batch analysis".

4. PLANNED ACTIVITIES - YEAR 02

Effects of exposure to microwaves on deoxyribonucleic acid (DNA) ribonucleic acid (RNA), and protein synthesis will be measured by the incorporation of methyl (^3H)-thymidine (^3H -TdR) (DNA substrate), ^3H -uridine (^3H -UR) (RNA substrate), and ^3H -leucine (protein substrate) by the mononuclear leukocytes in a microtiter system. [For a recent study of effects of microwaves using these assays in an animal model, see Wiktor-Jedrzejczak, W, et al: Bioelectromagnetics 1:161-170, 1980]. Spontaneous incorporation of tritiated substrates will be examined, as well as incorporation in response to stimulation. PHA will be used as a mitogenic stimulus. If effects of microwaves on response to PHA are observed, assays will be performed using other mitogens (eg, Con A, PWM) and antigens (eg, SKSD) to determine the generality of the effects.

Cytospin slides will be prepared for morphological analysis, specifically to detect any possible dissociation between blastogenesis determined by uptake of tritiated substrate and morphologic blastogenesis. Viability of cells will also be assayed by their ability to exclude trypan blue dye or ethidium bromide.

In initial studies of stimulated mononuclear leukocyte function, the cells will be exposed to microwaves during the first 24 hours of the three day incubation period used to determine transformation responsiveness to PHA. (Unstimulated responses will be determined concurrently.) It is in this time period that monocyte-macrophage function as an accessory cell and monocyte-lymphocyte interaction are occurring [see Roberts NJ, Jr. and Steigbigel RT: J. Immunol. 121:1052-1058, 1978]. Subsequent studies will examine responses with exposure of the cells to microwaves during periods after the triggering of lymphocyte processes has occurred, leading to DNA synthesis or transformation response. Initial studies will use 2450 MHz (CW) exposure for 120 minutes at low averaged SAR (≤ 0.5 to 1.0 mW/ml).

Additional details are to be found in the original Year 01 proposal.

APPENDIX

A Waveguide Exposure Facility for Examining Effects of
Microwaves on Human Leukocytes

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I. Facility:

Figure 1 shows the block diagram of the waveguide exposure facility for cell exposure to microwaves. The waveguide exposure chamber dimensions are shown in Figure 2. For initial studies (Sections II-VII), a stub tuner (as shown in Figure 1) and WR430 end plate were used. For subsequent studies, the tuner was removed and a WR430 waveguide-to-coaxial adapter and Hewlett-Packard Coaxial Termination (Model 908A) were used in place of the end plate. The studies were performed at 23°C ambient temperature.

II. Calorimetric determination of SAR:

- A. Theoretical considerations: [see Allis et al; Radio Sci. 12(suppl):1-8, 1977].

A system of mass m and heat capacity C , which initially is at temperature T_0 , is heated at a constant rate from time $t=0$ to time $t=\tau$, at which time heating is terminated. The temperature at any time will reflect a balance between input and loss of thermal energy. If we assume that the input is constant until time $t=\tau$ and for all time t , heat loss is proportional to the temperature difference between the temperature at any given time and the initial temperature ($\Delta T = T - T_0$) if the environment is held constant.

For heating:

$$dT/dt = A - k\Delta T \quad 0 \leq t \leq \tau \quad (1)$$

For cooling:

$$dT/dt = -k\Delta T \quad t \geq \tau \quad (2)$$

Equations (1) and (2) can be integrated to yield

$$\Delta T = A/k (1 - e^{-kt}) \quad 0 \leq t \leq \tau \quad (3)$$

$$\Delta T = \Delta T_{\tau} e^{-k(t-\tau)} \quad t \geq \tau \quad (4)$$

where A is the rate of increase in temperature in the absence of losses, and k is the proportionality constant for the rate of decrease in

temperature (cooling rate constant). If the temperature of the system is allowed to reach a steady state, the rate of energy input equals the rate of energy loss, and

$$\Delta T = \Delta T_{ss} = \text{constant.}$$

From equation (3), we find

$$\lim_{t \rightarrow \infty} \Delta T = \Delta T_{ss} = A/k \quad (5)$$

If the system is absorbing energy at a constant rate (W), then

$$W = mCA \quad (6)$$

The specific absorption rate (SAR) is the absorption rate per unit mass or volume; therefore,

$$\text{SAR} = W/m = CA \quad (7)$$

Substituting A from equation (5) into equation (7), we find

$$\text{SAR} = C \cdot \Delta T_{ss} \cdot k \quad (8)$$

From equation (4) at steady state, the cooling curve can be transformed into

$$\ln \Delta T = \ln \Delta T_{ss} - k(t - \tau) \quad (9)$$

From equation (5) at steady state, the heating curve can be transformed into

$$\ln (\Delta T_{ss} - \Delta T) = \ln \Delta T_{ss} - k \cdot t \quad (10)$$

Therefore, a semilogarithmic plot of equation 9 or 10

can yield an intercept (ΔT_{ss}) and a slope (k) for SAR determination.

B. Results and discussion:

Typical temperature tracings of different forward powers are shown in Figure 3. The temperature of the culture is measured with a microwave transparent Vitek 101 Electrothermia Monitor. A near-steady state temperature is achieved at the end of exposure. Analysis of SAR according to equations (9) and (10) shows a deviation from linearity usually occurring around 20 minutes for either heating analysis or cooling analysis. The deviation from linearity is partially attributed to digitalized data at

0.1°C intervals. Changes in the environment are also expected. The differences between SAR determination by heating and cooling are 0.58, 0.97, 1.19 and 1.47% in these examples.

However, the replication of SAR determination by heating and cooling is not a general rule. To obtain such replication, we have to maintain constant heat exchange between exposed sample and the environment. More than 20% difference can occur if caution is not exercised to maintain a constant heat exchange. For example, a relatively large cooling constant can be obtained by circulating air through the waveguide.

There is a low density styrofoam spacer in the present waveguide exposure facility, to maintain proper spacing of the plexiglass dram-vial holder. Therefore, the heat exchange in the exposed sample can be affected by the absorption and radiating characteristics of the air, waveguide and styrofoam, unless a jacket of medium to high heat capacity is used to buffer the variable exchange between exposed subject and air, air and styrofoam, air and waveguide, and waveguide and incubator environment.

Two observations indicate that heating analysis is superior to the cooling analysis in the present facility: (1) deviation from linearity occurs consistently earlier in the cooling curve than in the heating curve; and (2) a slower and larger deviation is noted in the cooling constant determined by cooling analysis than by heating analysis. The cooling constant is 0.0838 ± 0.0087 (S.D., $n=20$) from heating analysis and 0.0776 ± 0.093 (S.D., $n=19$) from cooling analysis. (One of the cooling curves was useless since the deviation from linearity occurred in less than 10 minutes.) The difficulties of cooling analysis are apparently attributable to the styrofoam spacer, which alters the heat exchange due to its insulation characteristics. In a relatively short period of time, the contribution of styrofoam is negligible since the temperature of styrofoam does not deviate much from the initial temperature.

A sixty minute exposure period and 40 minute cooling period was the standard procedure used in the determination of SAR. Specific heat of the medium was determined to be 0.97 cal/g/°C. Analysis of the heating curve is used for reasons stated above, and data are shown in Table I and Figure 4 (open circles). The correlation between SAR and forward power is highly significant. (The correlation coefficient is 0.95.) The regression coefficient is 4.24 mW/ml for every watt forward power, or 4.56 mW/ml for every mW forward power at sampling efficiency of -20.4 dB.

III. SAR determination by instrumentation:

The purpose of this study was to determine the SAR from the power reading sampled by the bidirectional coupler. Two criteria were used. These were SAR determination, which is not altered by changes in tuning of the system, and the result by instrument reading, which should be identical with the calorimetric procedure. The closed circles in Figure 4 indicate such achievement. The SAR determined by instrument reading is a compound function of the forward power (P_f) and reflected power (P_r). The data shown (Figure 4) are from four series of determinations with a change in tuning. The formula $[P_r \cdot (P_f - P_r) / (P_f + P_r) / \text{volume}]$, can provide a simple and accurate estimate of SAR in the present facility.

IV. SAR determination of the dual tube waveguide exposure facility:

The SAR is determined with the empirical formula, as indicated in Section III. Table II shows the results. Only minimal differences were noted between tubes. The largest difference was 0.56%.

V. Relationship between steady temperature increment and SAR:

Figure 5 shows the relationship between steady state temperature increment and SAR from 20 data points (obtained from Table I). The

regression coefficient is 0.2161 °C/mW/ml; the correlation coefficient is 0.98.

VI. Prediction of steady state temperature increment by the cooling constant:

From equation (5), we obtain

$$\Delta T_{ss} = 0.0147 \cdot SAR/k \quad (11)$$

The cooling constant has a dimension, min^{-1} , the SAR a dimension mW/ml .

The constant 0.0147 is a conversion factor for the units used. The 95% confidence limit of the cooling constant is 0.0655 to 0.1021 min^{-1} . Therefore, the steady state temperature increment is in the range of 0.2243 to 0.1440 °C/mW/ml. Experimental data are within this range. The range of cooling constant also predicts that the heating will be 98 to more than 99% complete by one hour of exposure.

VII. Calibration of the waveguide exposure system at different ambient temperatures:

A. Empirical calculation of SAR:

1. determine forward and reflected power at different forward powers;
2. calculate SAR according to $[P_r \cdot (P_f - P_r) / (P_f + P_r) / \text{volume}]$;
3. Plot and calculate the least-square-fit line for SAR versus forward power.

B. Prediction of steady state temperature increments at different ambient temperatures:

Expose the culture at a high forward power, to increase culture temperature by 5°C. Turn off the power. Record temperatures for a minimum of 15 minutes. Plot ΔT against time. Find the cooling constant k by the least-square-fit method according to equation (4). Use equation (11) to determine the range of steady state temperature increments.

From the SAR- P_f relationship, one can determine the forward power required for any particular SAR of interest. The P_r - P_f relationship is used to ascertain that there is no drift in the system. The ΔT_{ss} at one hour exposure is the final check against malfunction of the exposure system and environmental control.

VIII. Dosimetry using a muscle phantom:

For these and subsequent studies, a WR430 waveguide-to-coaxial adapter and coaxial termination were substituted for the end plate.

The muscle phantom was prepared according to the formula provided by Guy [IEEE Trans. MTT 19:205-214, 1971]. Two cut vials of 1.2 cm diameter and 1.6 cm height were used for these determinations. These vials were filled with muscle phantom without air bubbles. A 5x6 grid (30 points) was used. (Points of measurement are indicated in Figure 6). Landmarks for these 30 points were the center of the vial, and 0.5 cm to the front (proximal), 0.5 cm to the back (distal), 0.5 cm to the right and 0.5 cm to the left from center of the vial. A 0.3 cm incremental spacing from bottom of the vial was used to determine 6 points of measurement for each of the 5 vertical columns. A 6 second exposure time was used for each determination, with triplicate measurements. Temperature was measured with a Vitek non-perturbing thermosensor. Signals from this sensor were fed into a linear recorder with a deflection of 0.1°C/inch. The rate of heating ($d\Delta T/dt$) was determined from this tracing. The temperature tracing was usually linear with time, except at the highest SAR's. In these cases, a linear portion of the first 1.5-3.5 seconds of exposure was used to avoid the initial overshoot of the power generating equipment, and the rate of heating was used to calculate SAR. A forward power of 0.55 watts was used throughout these determinations.

The averaged SAR was 58.7 ± 46.2 (S.D., $n=30$), and 63.4 ± 45.4 ($n=29$) in the right vial in two series of determinations, and 76.1 ± 63.9 ($n=30$) in the left vial. The ratio of the SAR at each point to the averaged SAR in the given vial ranged from 0.12 to 3.94 for the right vial and 0.24 to 3.39 for the left vial. These ratios were plotted in planes (Figure 7). A geometric resonance was apparent. Maximal absorption occurred approximately in the center of each vial.

The correlation between points in the duplicate series of determinations using the right vial was highly significant (correlation coefficient = 0.7471, $P < 0.001$). (Replication of each exact location for measurement was difficult, preventing identical determinations from the two sets. Averaged SAR values did approximate each other).

IX. Effect of Agitation on Temperature Tracings in the Culture Medium:

Two points were selected according to the SAR distribution determined previously. These points were the highest SAR (300 mW/ml) and lowest SAR (9 mW/ml), with an averaged SAR of 76 mW/ml within the vial. Figure 8 indicates the temperature tracing for these points with or without agitation. (Agitation is provided by an Eberback Shaker located external to the incubator holding the waveguide, and attached to the movable platform on which the waveguide is placed). Comparison among these tracings without agitation revealed a thermal gradient, and heat transfer from the high SAR to the low SAR point. The thermal gradient was reduced and maintained at approximately 0.1°C with agitation. With agitation, the change in rate of heating became insignificant after 10 seconds of exposure. At a lower absorption rate, the temperature inhomogeneity is probably insignificant.

X. Determination of the averaged SAR by calorimetric methods:

A. Calorimetric procedure with low density styrofoam for thermal insulation:

To minimize temperature dependent heat loss in the test material, a low density styrofoam was used. For a representative value of the averaged SAR, a point within the vial was selected for measurement when heat loss was negligible. Criteria used to select that point were:

(1) the heating curve for the last 15 seconds of the 20 second exposure was linear; (2) the temperature increment during the exposure did not exceed 0.2°C ; and (3) after termination of exposure, the temperature changes in the first 5 seconds did not exceed 0.01°C . A forward power of 0.55 watts was used. The averaged SAR thus determined was 34.1 ± 2.86 (S.D., $n=4$) in the right vial, and 31.3 ± 3.16 mW/ml (S.D., $n=4$) in the left vial,

B. Non-steady state calorimetric study:

Heating characteristics of the medium can be represented by $\Delta T = A/k (1 - e^{-kt})$ and cooling characteristics by $\Delta T = \Delta T_e e^{-kt}$.

Culture medium was exposed to 0.55 watts forward power for 20 seconds with agitation. The cooling constant, k , was determined within 20 seconds after exposure. The k was then used to determine the absorption rate, A , by heating equation. The SAR thus determined were 80 ± 23 (S.D., $n=4$) in the right vial, and 73 ± 9 (S.D., $n=4$) in the left vial.

C. Steady-state calorimetric study:

According to Ali et al [Radio Sci 12(suppl):1-8, 1977], the heating curve can be reduced to the equation: $\ln (\Delta T_{ss} - \Delta T) = -kt + \ln \Delta T_{ss}$. The ΔT_{ss} is the steady state temperature increment. The cooling constant, k (-0.0938 min^{-1}), was determined previously. The steady-state temperature can be determined from this constant, k ; i.e., 99% of steady-state temperature increment can be achieved in 55 minutes of exposure.

The ΔT_{ss} was determined in culture medium with agitation after 60 minutes of continuous exposure. The initial 15 to 20 minutes of the heating curve was used. The temperature increment (ΔT) of every 30-second period was digitalized for calculation of k and ΔT_{ss} . Forward power was set at 0.55 watts. Results indicated that the right vial absorbed at 26.4 ± 4.4 (S.D., $n=4$) and the left vial at 28.8 ± 0.67 mW/ml (S.D., $n=4$).

XI. Discussion and Summary:

The findings are summarized in Table III and Figure 9. Inhomogeneity and significant resonance absorption were noted in the dual vial waveguide exposure facility. A 30-point measurement of SAR distribution revealed that the SAR at any of the measured points could range between 0.12- and 3.94-fold of the averaged SAR within the given vial. However, such variable SARs did not create significant thermal gradients within vials when external agitation was applied. The differences in averaged SAR between vials of the present dual-vial waveguide system were not significant, irrespective of the method of quantitation used.

Depending upon the method used, the averaged SAR of a vial could be segregated into two values. There was a two-fold difference between these values.

Incident power density is probably the only reasonable measurement for judging environmental contamination and for determining personnel protection guidelines. Extrapolation from in vitro experiments to an in vivo biological effect can be achieved by converting the SAR in waveguide-exposed biological material to an equivalent incident power density in vivo. Therefore, using the lower value would provide a margin of safety if a threshold SAR for biological experimentation can be determined.

The difference between maximum and minimum SAR within a culture vial was 28-fold. However, the difference in thermal environments at these points was negligibly small; in addition, studies with much lower averaged SAR are planned. A study of biological parameter-temperature relationships, in microwave-exposed and sham-exposed leukocyte cultures using the present facility can provide an assessment of the importance of inhomogeneous SAR on a biological system without substantial compromise by thermal mechanisms.

TABLE I
CALORIMETRIC DETERMINATION OF SAR USING HEATING ANALYSIS

<u>Forward Power (W)</u>	<u>$\Delta T_{ss} (^{\circ}C)^a$</u>	<u>$k (min^{-1})^b$</u>	<u>SAR (mW/ml)^c</u>
0.22	2.28	-0.0617	9.54
0.33	3.04	-0.0928	15.96
0.45	3.47	-0.0710	16.70
0.55	4.91	-0.0794	26.49
0.55	4.53	-0.0961	29.57
0.67	4.79	-0.0751	24.45
0.67	4.57	-0.0921	28.59
0.77	6.98	-0.0763	35.68
0.78	6.44	-0.0866	37.39
0.78	5.63	-0.0824	31.48
0.79	5.10	-0.0864	29.92
0.89	7.24	-0.0798	39.22
0.89	6.40	-0.0841	36.54
1.00	6.60	-0.0733	35.12
1.01	7.03	-0.0882	42.10
1.01	7.43	-0.0942	47.54
1.10	6.40	-0.0902	39.20
1.13	8.10	-0.0896	49.26
1.18	9.24	-0.0809	50.78
1.28	9.39	-0.0909	57.95

-0.0838

± 0.0087 (mean \pm S.D.)

^a ΔT_{ss} = steady state temperature increment

^b k = cooling constant

^c SAR: specific absorption rate

TABLE II
COMPARISON OF ABSORPTION RATES OF RIGHT AND LEFT VIALS

<u>Forward Power</u> <u>(mW -20.4 dB)</u>	<u>SAR, Left Vial</u>	<u>SAR, Right Vial</u>
0.1	0.93	0.93
0.5	4.70	4.69
0.8	7.51	7.50
1.0	9.40	9.38
1.5	14.09	14.08
1.8	16.92	16.88
2.0	18.80	18.78
2.5	23.51	23.46
2.8	--	26.30
2.85	26.80	--
3.0	28.21	28.17
3.5	32.91	32.89
3.8	35.74	35.71
4.0	37.61	37.60
5.0	--	47.03
5.1	47.97	--
6.0	--	65.43
6.1	57.38	--
7.0	65.84	--
7.2	--	67.73
8.3	--	78.07
8.4	78.98	--
9.2	86.48	86.54
10.0	93.98	94.06

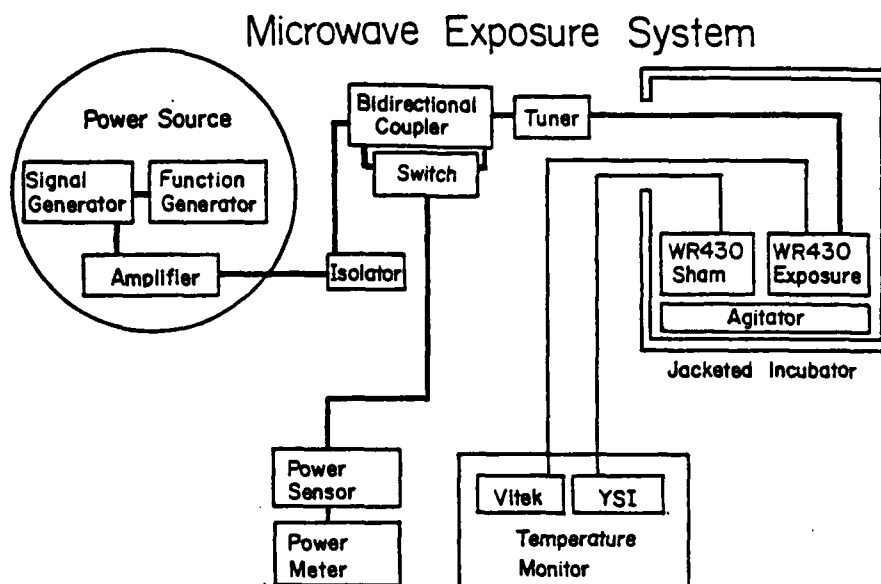
TABLE III
DETERMINATIONS OF SAR WITH DIFFERENT DOSIMETRIC PROCEDURES

Procedure	Right Vial	Left Vial
Muscle phantom ^a ,	58.7 \pm 46.2 (30) ^b	76.1 \pm 63.9 (30) ^b
Lim d/dt (ΔT)	63.4 \pm 45.4 (29)	
t=0		
Culture medium, Agitated,	80.0 \pm 23.1 (4)	72.9 \pm 9.2 (4)
Non-insulated, Non-steady-state		
Culture medium, Agitated,	34.1 \pm 2.9 (4)	31.3 \pm 3.2 (4)
Insulated, Linear heating rate		
Culture medium, Agitated,	26.4 \pm 4.4 (4)	28.8 \pm 0.7 (4)
Non-insulated, Steady-state		

a = Multiple points measured within the vial.

b = Mean \pm S.D. (No. of determinations).

Figure 1

COMPONENTS

Power Source:

1. Hewlett-Packard Signal Generator, HP8616A
2. Krohn-Hite Function Generator, 5500 AR
3. Hughes Amplifier, 1177-01

Delivery:

1. Andrew Superflexible Heliax 1/4 in. coaxial cable
2. Harris Isolator, PRD 1211C
3. Weinschel Stub Tuner, DS 109
4. Omega Waveguide, WR 430
5. Hewlett-Packard Coaxial Termination, 908A

Power Measurement:

1. Narda Bidirectional Coupler, 3022
2. Sage Lab. Switch, STN 2180A Type 1P2T
3. Hewlett Packard Power Sensor, 8478B
4. Hewlett Packard Power Meter, 4328

Temperature Measurement:

1. Vitek 101 Electrothermia Probe and Monitor
2. YSI 520 Tissue Implantable Probe
3. Markson Bridge, T-16458

Other:

1. National Water-Jacketed Incubator, 7241, Modified
2. Eberbach Shaker, Variable Speed, 5850

WAVEGUIDE EXPOSURE CHAMBER

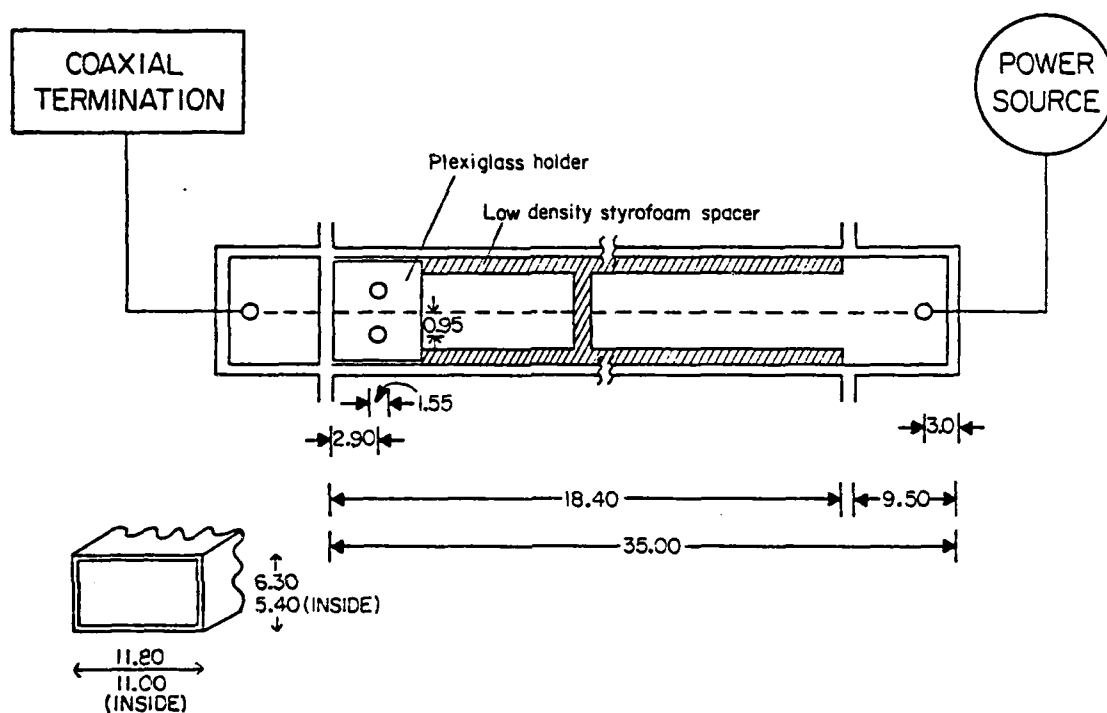
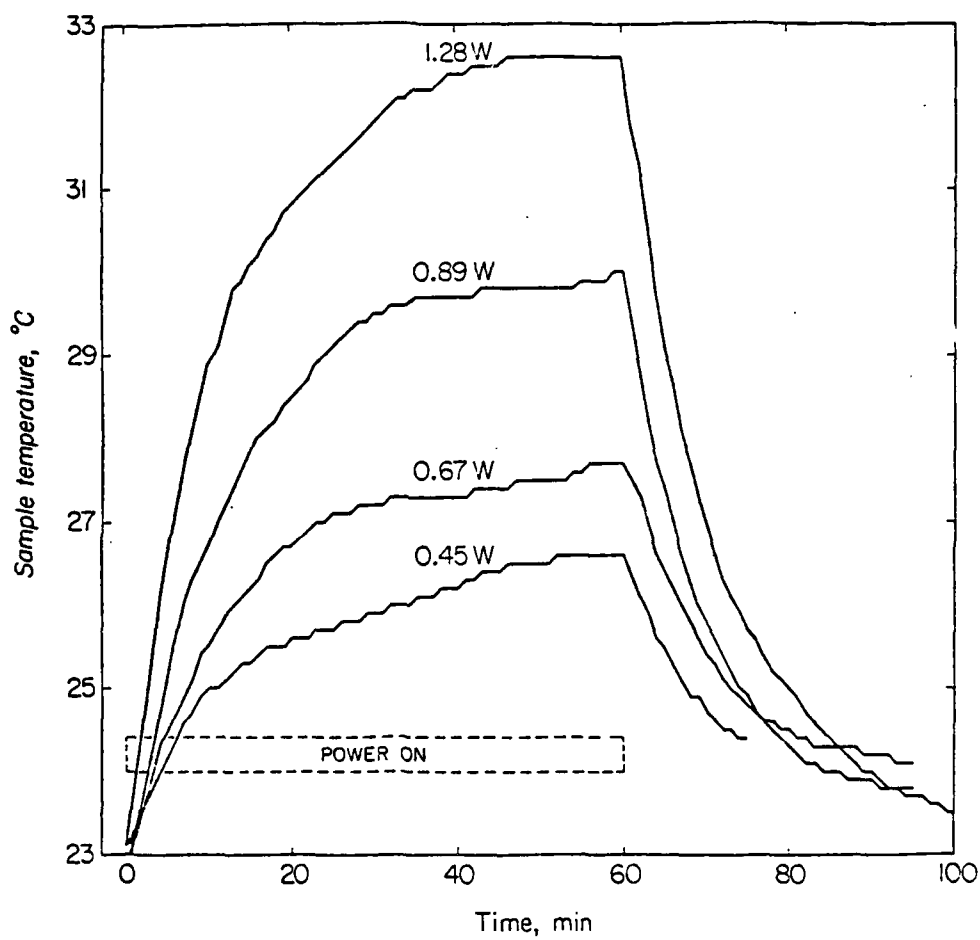


Figure 2. Waveguide exposure chamber
(Dimensions are in cm.)

Figure 3

TEMPERATURE TRACINGS AT DIFFERENT
FORWARD POWERS ($T_{\text{air}} = 24^{\circ}\text{C}$)



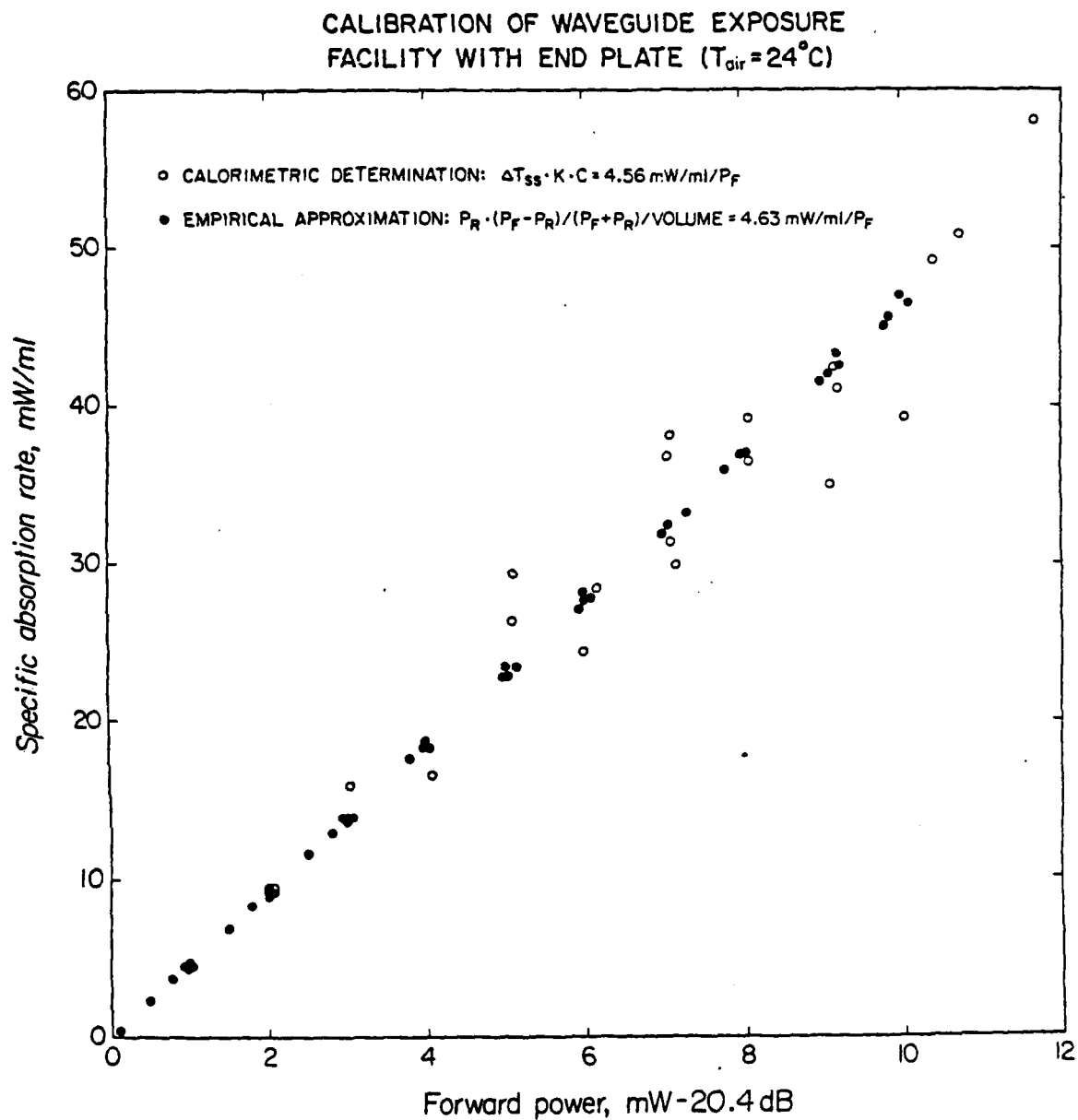


Figure 4. Calibration of waveguide exposure facility with end plate. ($T_{air} = 24^{\circ}C$.)

STEADY-STATE TEMPERATURE INCREMENT IN WAVEGUIDE EXPOSURE FACILITY ($T_{\text{air}}=24^{\circ}\text{C}$)

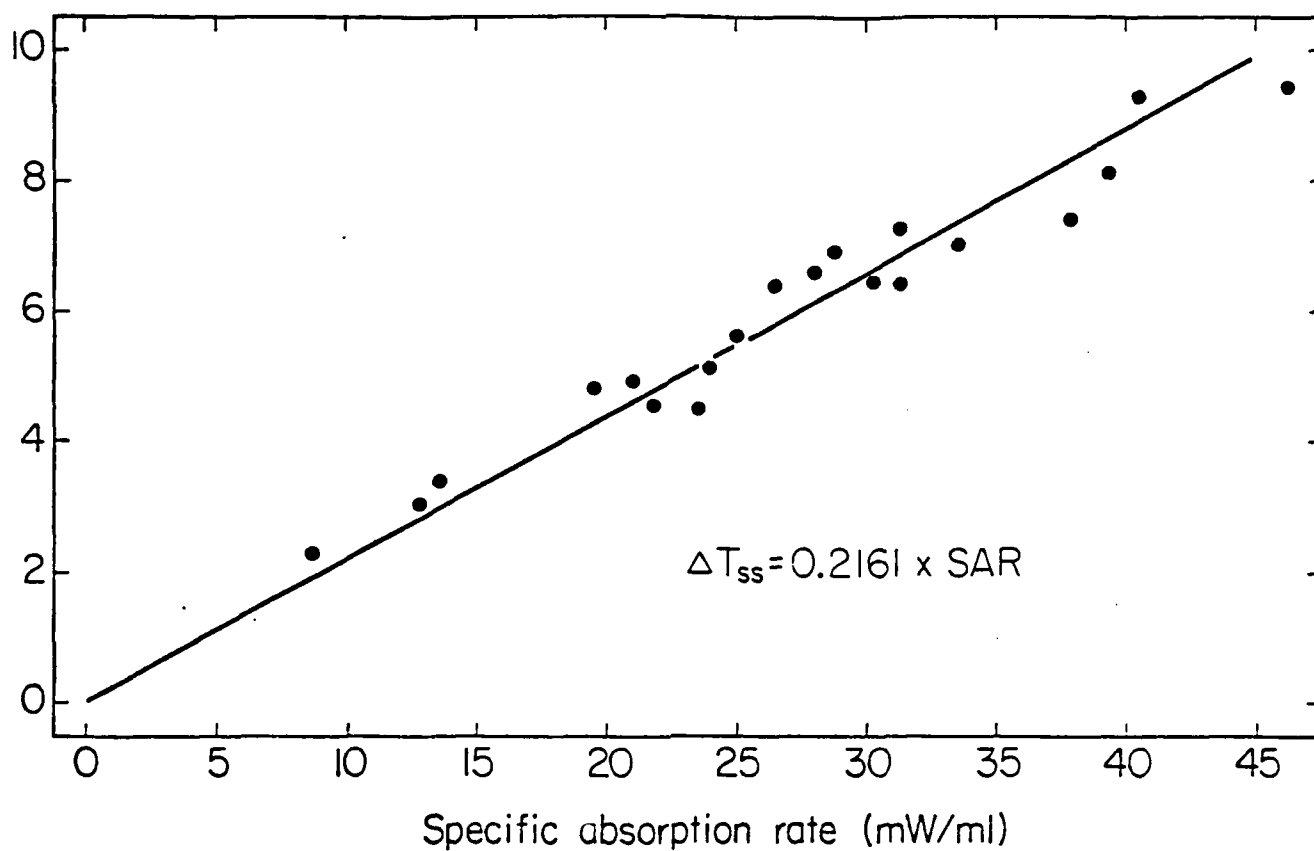
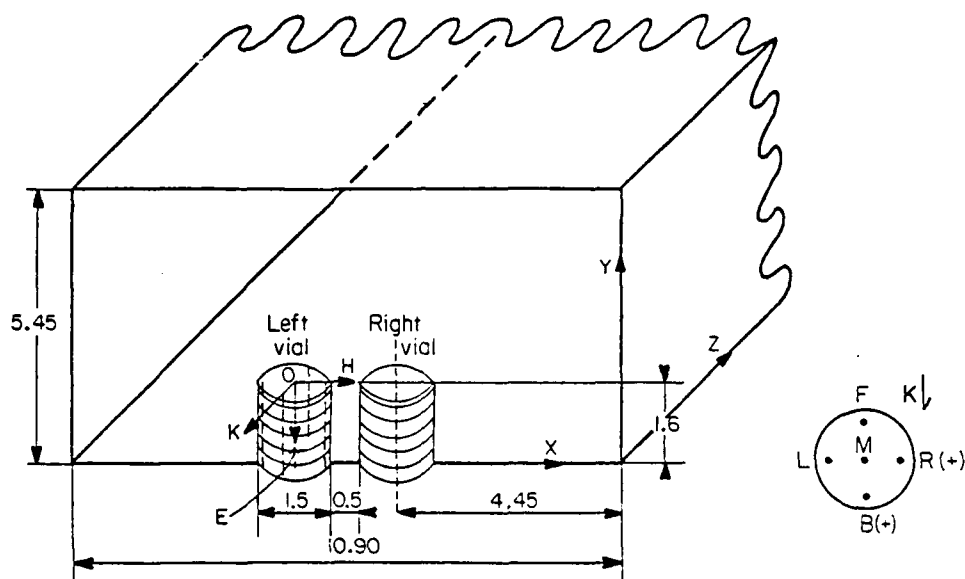


Figure 5. Steady-state temperature increment in waveguide exposure facility. ($T_{\text{air}} = 24^{\circ}\text{C}.$)

Figure 6

LOCATIONS WITHIN CULTURE VIAL
USED FOR DETERMINATION OF SAR PROFILE

Dimensions are in cm.

SAR PROFILE IN MUSCLE PHANTOM

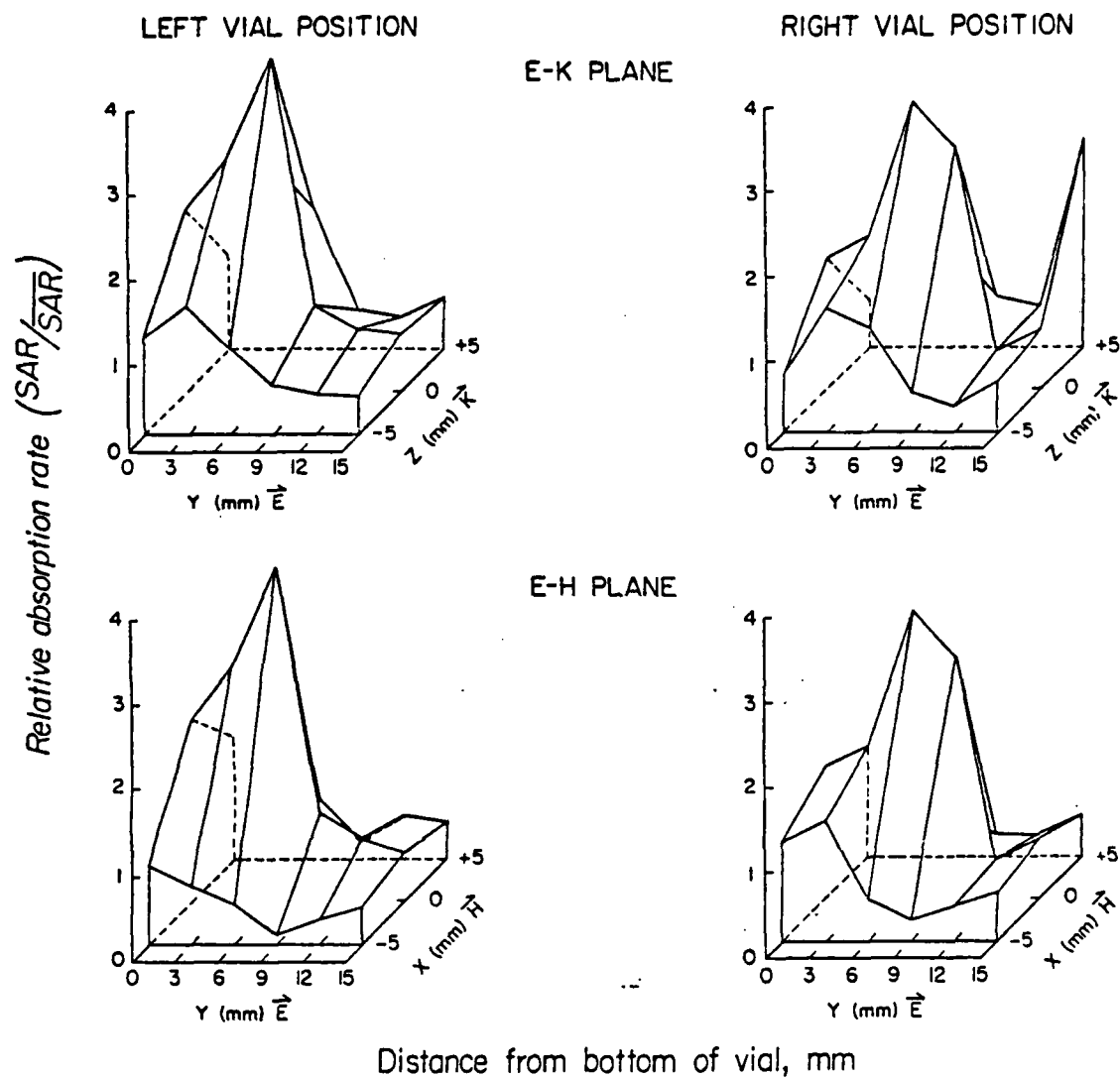


Figure 7. SAR profile in muscle phantom.
 Forward power = 0.55W, $N = 30$.
 Left vial average $\text{SAR} \pm \text{S.D.} = 76 \pm 64 \text{ mW/ml}$.
 Right vial average $\text{SAR} \pm \text{S.D.} = 59 \pm 46 \text{ mW/ml}$.

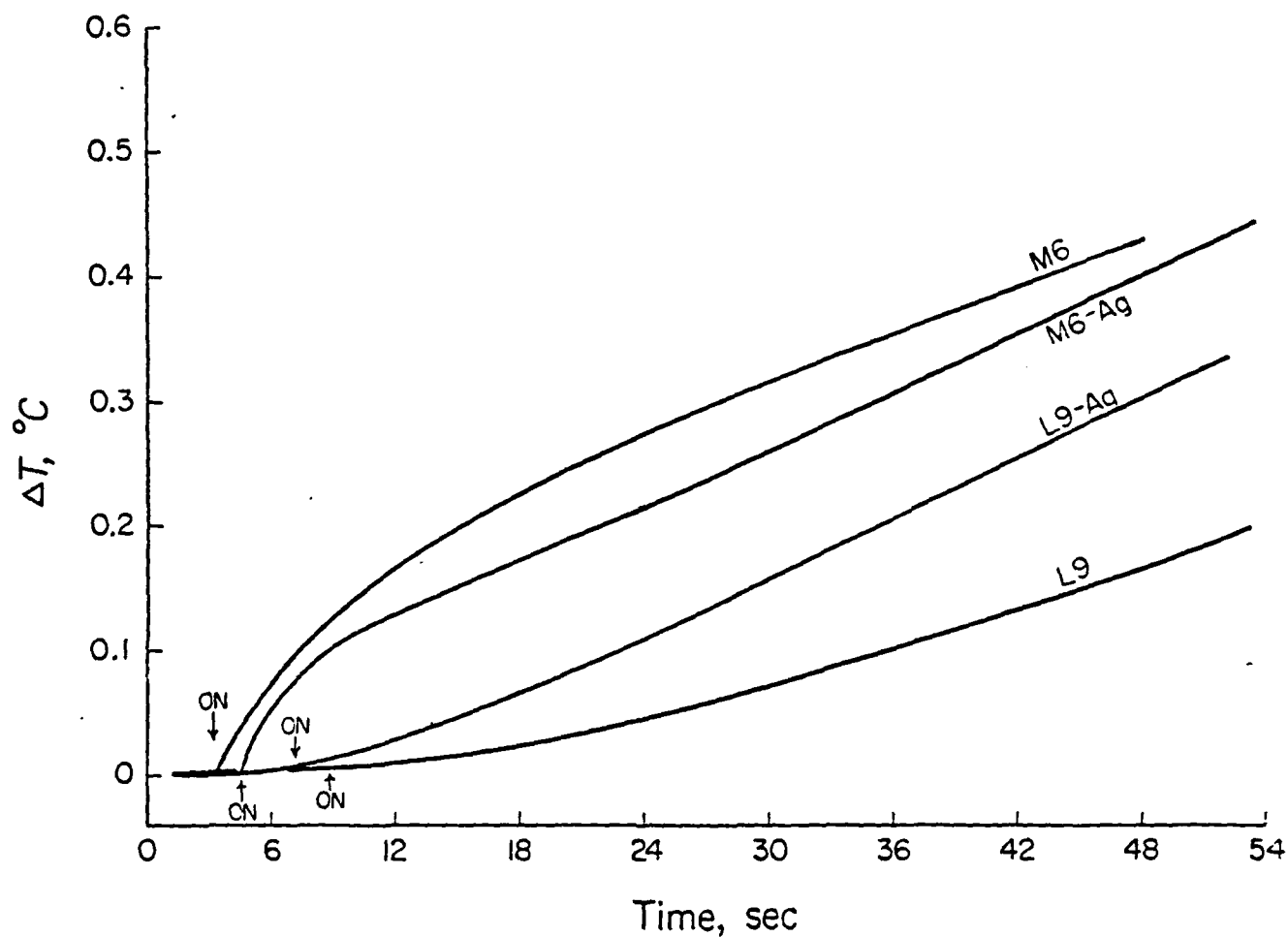
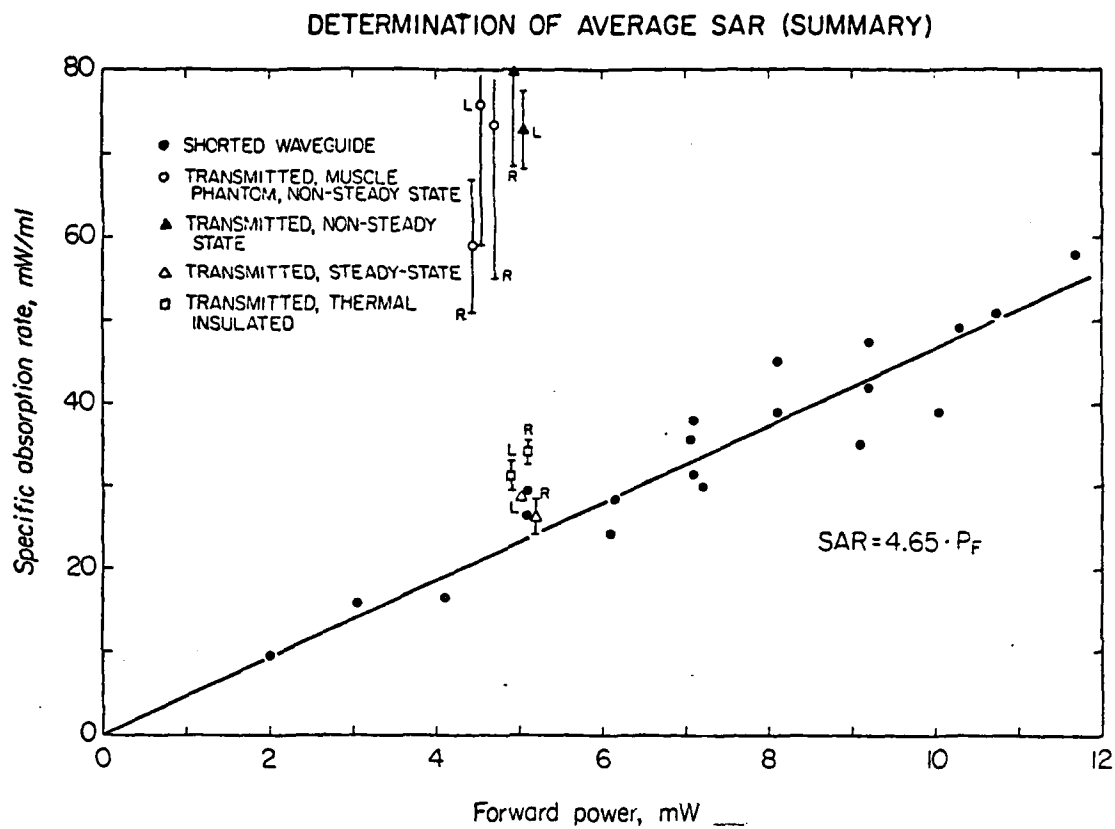
EFFECT OF AGITATION ON TEMPERATURE
TRACINGS OF EXPOSED SAMPLE

Figure 8. Effect of agitation on temperature tracings of exposed sample. L9 is the point of lowest SAR and M6 is the point of highest SAR (see Figure 7). Ag indicates that agitation was applied.

Figure 9



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REPORT DOCUMENTATION PAGE		AD INSTRUCTIONS BEFORE COMPLETING FORM	
1. REPORT NUMBER AFOSR-TR- 81 -0537	2. GOVT ACCESSION NO AD-A101 081	3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) Microwaves and Human Leukocyte Function		5. TYPE OF REPORT & PERIOD COVERED Final	
		6. PERFORMING ORG. REPORT NUMBER	
7. AUTHOR(s) Norbert J. Roberts, Jr., M.D.		8. CONTRACT OR GRANT NUMBER(s) AFOSR-80-0111	
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Rochester School of Medicine 601 Elmwood Avenue Rochester, New York 14642		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102F 2312/A5	
11. CONTROLLING OFFICE NAME AND ADDRESS Air Force Office of Scientific Research/NL Bolling AFB, DC 20332		12. REPORT DATE February 19, 1981	
		13. NUMBER OF PAGES 104	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified	
		15a. DECLASSIFICATION DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited.			
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)			
18. SUPPLEMENTARY NOTES			
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Microwaves, human leukocytes			
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The objective of these studies is to determine whether exposure to micro- waves(2450 MHz) affects the function of human leukocytes in the resting state and during antigenic or mitogenic challenge. This paper is a summary report of the construction and calibration of a waveguide exposure system for the exposure of human mononuclear leukocytes to 2450 MHz (CM) microwave energy. A description of the dual vial waveguide exposure facility for in vitro irradiation of human leukocytes is presented. Calorimetric determinations of specific absorption rates (SAR) were made using heating curves measured with			

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

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20. ABSTRACT (continued)

- a microwave transparent Vitek 101 Electrothermia Monitor. The correlation between SAR and forward power was highly significant ($r = 0.95$). At a forward power of 0.55 W the averaged SAR was approximately 33 mW/ml. However, inhomogeneity and significant resonance absorption were noted in the dual vial waveguide exposure facility. A 30-point measurement of SAR distribution revealed that the SAR at any of the measured points could range between 0.12- and 3.94-fold of the average SAR within the given vial. Measurements indicated that this variability in SAR values did not create significant thermal gradients within the vials when external agitation was applied.